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Synthesis, theoretical and biological studies of some novel Monoterpenic thiazolidin-4-ones

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Abstract: *New sulfur and nitrogen containing heterocycles with monoterpenic skeleton were prepared starting from the corresponding monoterpenic thiosemicarbazones. Chloroacetic acid or ethyl bromoacetate were used to have 2-substituted thiazolidin-4-ones beside indazole **8** resulting from pulegone thiosemicarbazone rearrangement in both acidic or basic media. The structures of the newly obtained compounds were confirmed by analytical mass and NMR spectra, as well as DFT calculations. All synthesized compounds were tested against fungi (Aspergillus fumigatus), yeast (Candida albicans), gram-negative (Acinetobacter baumannii and Pseudomonas aeruginosa) and gram-positive bacteria (Meticillin-resistant Staphylococcus aureus MRSA). The activities were tested at different concentrations. All compounds showed good antifungal but low antibacterial activities.*

Keywords: *Thiosemicarbazones, monoterpenic thiazolidin-4-one, Indazole, heterocyclisation, DFT calculation, antibacterial and antifungal activities, MIC.*

I. Introduction

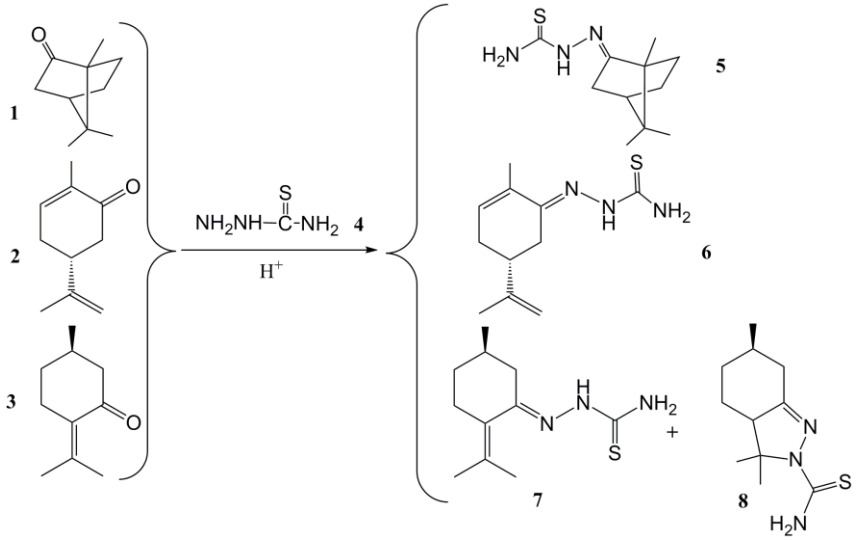
In the recent literature, a growing interest for heterocyclic systems due to their applications in many different fields such as biology, pharmacology or industrial chemistry. Indeed many five membered heterocycles containing sulfur and nitrogen, such as thiazolidinones are known to possess a broad spectrum of biological activities such as anticonvulsants [1] anti HIV [2-4] antimicrobial [5-7] anti-inflammatory and anti-cancer [8-9]. In view of the above mentioned interesting properties and in continuation of our effort [10] to prepare new heterocyclic systems with improved biological activities, we report herein the synthesis of some new thiazolidin-4-one derivatives using heterocyclization reactions. (1*R*)-Camphor, (*R*)-carvone and (*R*)-pulegone thiosemicarbazones, have been transformed and the antimicrobial activity of the resulting products was determined.

II. Results and discussion

2.1 Chemistry

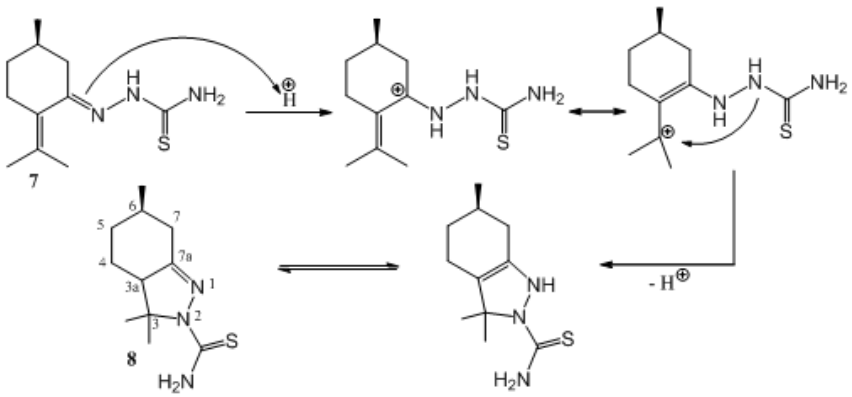
The first step in the synthesis of the desired thiazolidin-4-ones is the synthesis of thiosemicarbazones **5**-**7**, via the condensation of thiosemicarbazide **4** with the corresponding monoterpenes **1**-**3** in the presence of traces of sulfuric acid. While thiosemicarbazones **5** and **6** were obtained in high yields (81% and 88%), pulegone thiosemicarbazone **7** was isolated in low yield (6%) concomitantly with the rearrangement product **8** (84%) as major product of the reaction (scheme-1).

Scheme-1 Thiosemicarbazone **5-7** and indazole **8** preparation:

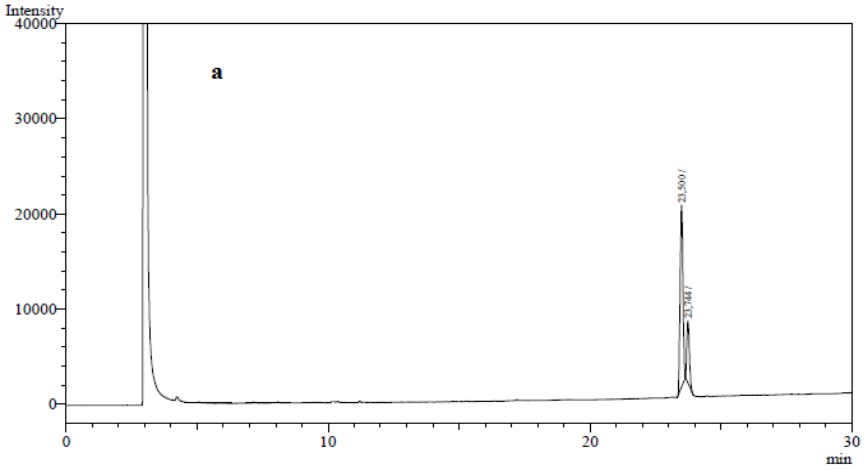


Most probably, compound **8** is formed from **7**, by acid catalyzed intramolecular addition of Hydrazonic N-H bond to C=C double bond as shown in (scheme-2).

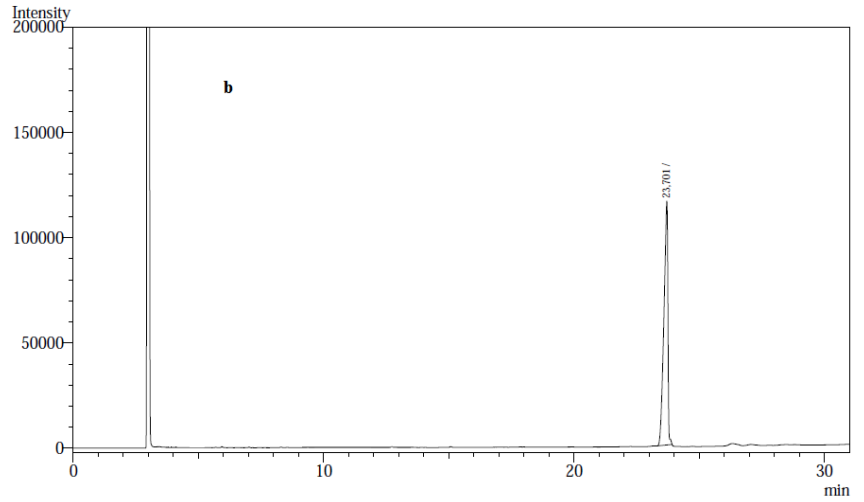
Scheme-2 Mechanism of thiosemicarbazone **7** rearrangement into indazole **8** in acidic medium:



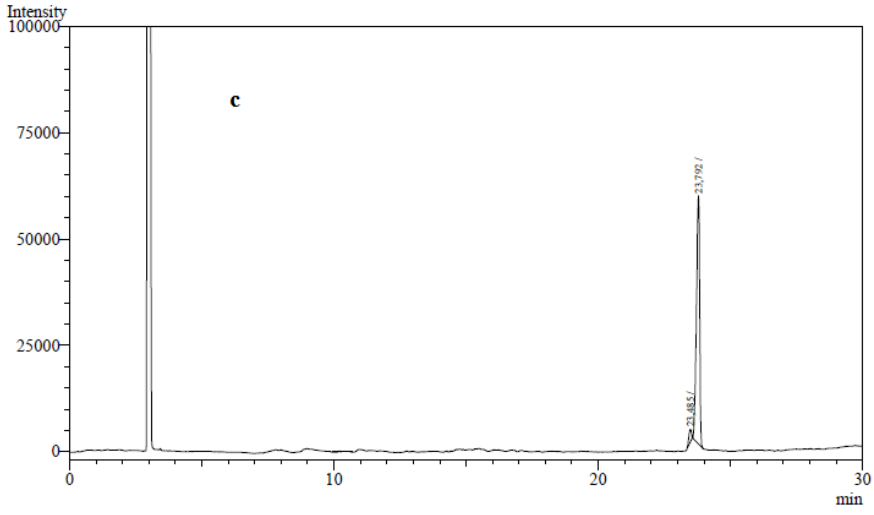
To avoid this rearrangement, we have prepared **7** in good yield (86%) in the absence of acid, by heating ethanol solution of equimolar quantities of pulegone and Thiosemicarbazide [11]. The structures of the thiosemicarbazones **5-7** were determined on the basis of their spectral data. Especially, the tautomeric form on the thiocarbamide moiety (-NH-C(SH)=NH) was characterized using ^{1}H and ^{13}C NMR spectra. Three broad singlets between 6.5-9.2 ppm and a quaternary carbon signal at about 180 ppm were thus attributed respectively. All the spectral data of **5-7** were in good accordance with the literature [11-13]. The structure of the newly isolated pyrazole **8** was determined by its mass and NMR (1D & 2D) spectral data. Indeed, the HRMS spectrum of **8**, shows molecular ion ($M+H^{+}$) at m/z=226.1371 corroborating its molecular formula $C_{11}H_{19}N_3S$. The ^{1}H NMR spectrum exhibits only two broad signals at 5.78 and 6.94 ppm due to protons of tautomeric form of the carbamothioyl group -CS-NH_2. The three methyl groups resonate respectively as a doublet (J=7.2Hz) at 0.89 ppm and two singlets at 1.66 and 1.80 ppm. We also note a multiplet at 2.66 ppm ascribable to the proton at C3a position. In ^{13}C NMR spectroscopy, the lack of signals at 126.2 and 131.0 ppm due to ethylenic quaternary carbons and the appearance of two splitted signals at 57.87; 59.07 ppm (C3a) and 66.56; 67.62 ppm (C3) provided a firm support for compound **8** structure. We also note, a splitting for signal due to C7a carbon (158.9; 159.81 ppm) showing that **8** was obtained as a diastereomeric mixture. This was confirmed by GC which revealed 76/24 ratio for the two diastereoisomers **8a**/**8b** (Fig. 1**a**). The two diastereoisomers **8a** (3aR,6R) and **8b** (3aS,6R) were then separated by liquid chromatography using hexane/ethyl acetate 9/1 as eluent followed by repetitive fractionated crystallization from ethanol (Fig 1). While **8b** was isolated as non-transparent powder, **8a** was separated as transparent crystals.



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	23,500	142942	19083	76,415				
2	23,744	44117	6524	23,585				
Total		187059	25607					



Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	23,701	1243083	115728	100,000				
Total		1243083	115728					

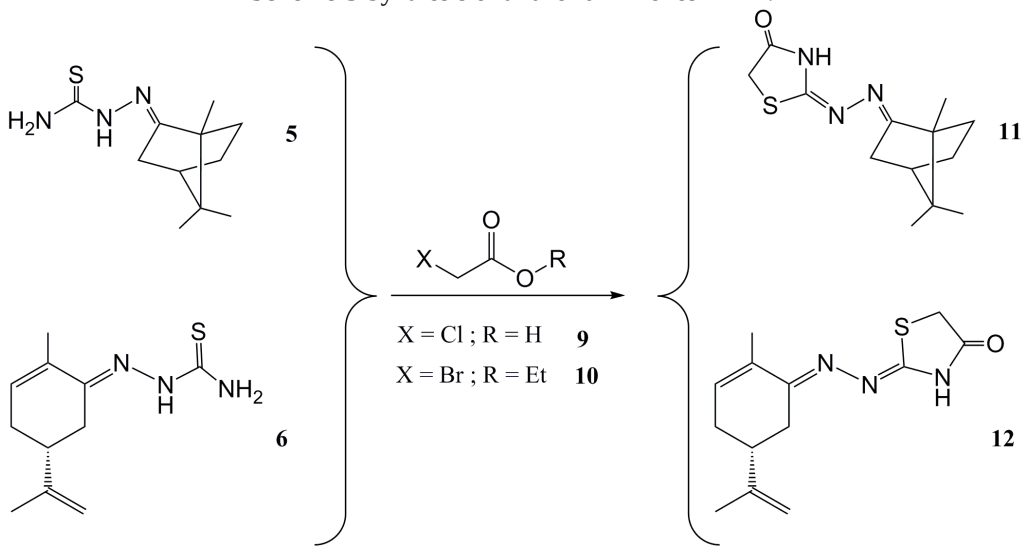


Peak#	Ret.Time	Area	Height	Conc.	Unit	Mark	ID#	Cmpd Name
1	23,485	20137	2958	3,899				
2	23,792	496377	58300	96,101				
Total		516514	61258					

Figure 1 GC analysis of diastereoisomeric mixture **8** (**a**), separated diastereoisomer **8a** (**b**) and separated diastereoisomer **8b** (**c**)

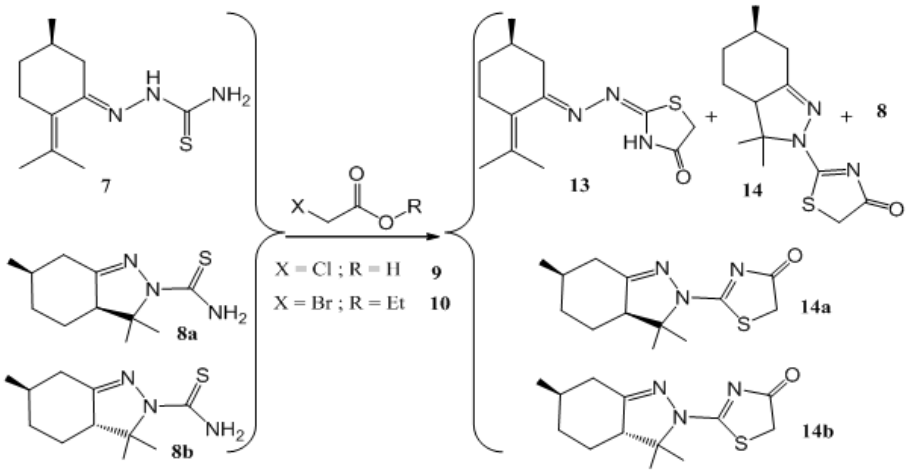
The second step of the desired thiazolidin-4-ones synthesis was the heterocyclisation of the thiosemicarbazones **5-7** and the two indazolecarbothioamides **8a-b**, with chloracetic acid, according to a previously described procedure [14-15]. The reaction was performed in ethanol under refluxing conditions giving respectively, thiazolidin-4-ones **11**, **12**, **14a** and **14b** (schemes-3,4) for **5**, **6**, **8a** and **8b** with good yields (**11:** 70%; **12:** 78%; **14a**: 60%; **14b**: 60%).

Scheme-3 Synthesis of thiazolidin-4-ones **11-12**:



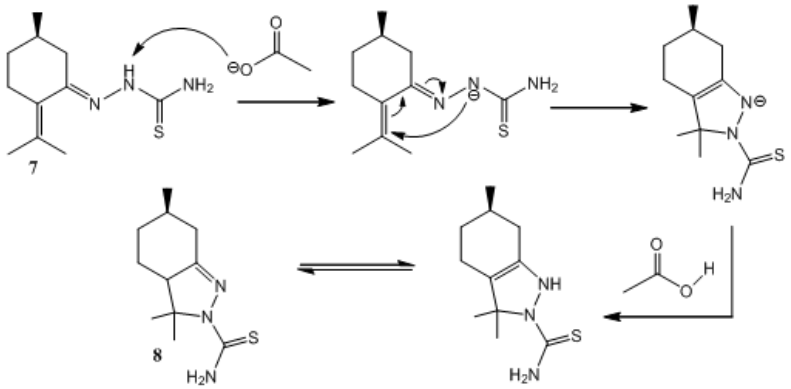
In contrast, the reaction of thiosemicarbazone **7** afforded the expected thiazolidin-4-ones **13** and compound **8** with low yields (**13:** 9%; **8:** 28%) concomitantly with pyrazolo-thiazolidin-4-one **14** (which is a diastereoisomeric mixture of **14a** and **14b**) as the major product (**14:** 60%) (scheme-4).

Scheme-4 Synthesis of thiazolidin-4-ones **13** and **14b**:



These results suggest that **7** underwent two competing reactions. The first, which is very fast, is the rearrangement of **7** into **8** (in an acidic medium) before a subsequent heterocyclisation reaction with chloracetic acid providing mainly pyrazolo-thiazolidin-4-one **14** takes place. The second reaction, relatively slow, is a direct heterocyclisation of **7** giving **13** with low yield. In view to improve the yield of thiazolidin-4-ones **11-13** and/or to prepare new heterocyclic systems with monoterpenic skeleton, we have tested the heterocyclisation reaction of thiosemicarbazones **5-7** and the two separated indazolecarbothioamides **8a** and **8b,** with ethyl bromoacetate **10** as cyclizing agent (schemes-3 and 4). The reaction was carried out in boiling absolute ethanol containing three equivalents of anhydrous sodium acetate to furnish the same results as the heterocyclisation reaction with chloracetic acid. However, we note a slight yield improvement for **11** (82%), **12** (86%), **14** (65%), **14a** (90%), **14b** (90%). Here, it's noteworthy to emphasize that obtaining **14** from **7**, would be explained by heterocyclisation of **8** formed after catalyzed addition of hydrazonic N-H bond to C=C double bond under basic conditions as shown in (scheme-5).

Scheme-5 Mechanism of thiosemicarbazone **7** rearrangement into indazole **8** in basic medium:



All the newly obtained heterocycles **11-14** were fully characterized by their spectroscopic data and clearly identified as thiazolidin-4-one derivatives by the appearance of a typical singlet in ^{1}H NMR spectra at about 3.7 ppm (**11:** 3.71 ppm, **12:** 3.74 ppm, **13:** 3.71 ppm, **14a-b:** 3.77 ppm) assigned to methylene protons of the thiazolidinone nucleus and the observation of distinctive ^{13}C NMR resonances in the regions 33-39 ppm (**11:** 33.22 ppm, **12:** 33.15 ppm, **13:** 34.86 ppm, **14a-b:** 38.09 ppm assigned to methylene carbon of the thiazolidinone nucleus) and 170-189 ppm (**11:** 174.28 ppm, **12:** 174.00 ppm, **13:** 170.16 ppm, **14a-b:** 188.48 ppm assigned to C=O of the thiazolidinone nucleus). Especially for **14**, we note splitted signals mainly for C3a carbon (58.88; 59.08 ppm) and for indazole C=N group (165.67; 166.86 ppm) which indicates that **14** was obtained as a diastereoisomeric mixture (**14a** and **14b**). The absolute configurations of **8a**, **8b**, **14a** and **14b** were determined respectively as (3aR, 6R), (3aS, 6R), (3aR, 6R) and (3aS, 6R) based mainly on the synthetic pathway and, implied by an X-ray analysis curried out on monocrystals of **14b** (Fig. 2) [16].

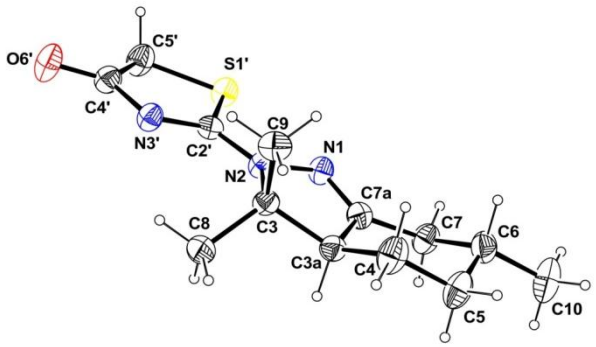
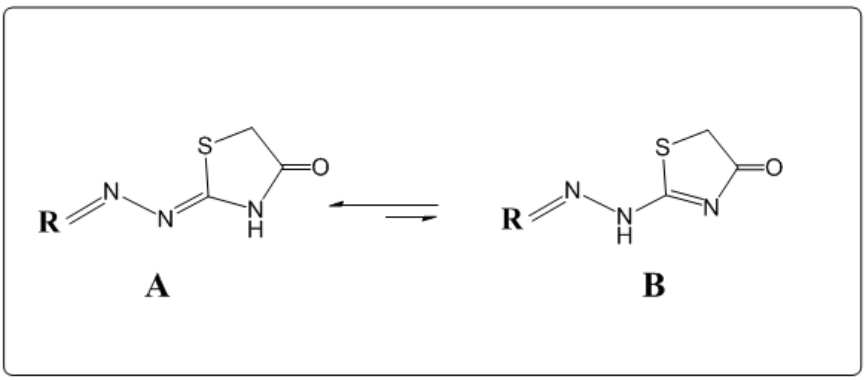


Figure 2 ORTEP view of the molecular structure of **14b** with atoms labelling scheme. Ellipsoids are drawn at 50% probability.

However, literature survey reveals that thiazolidin-4-ones with a nitrogen atom in alpha position exist as two tautomeric forms: 2-iminothiazolidin-4-one (form **A**) and 2-aminothiazolidin-4-one (form **B**) [17] (scheme-6).

Scheme-6 A and **B** possible tautomeric forms of Thiazolidin-4-ones



2.2 Theoretical Study.

In an attempt to determine which of the two tautomeric forms **A** or **B** is thermodynamically predominant, a DFT calculation was performed using the Gaussian 09W program system [18]. The geometry optimization and vibrational frequency calculations were performed at the B3LYP/6-311+G(d,p) level [19]. No symmetry constraints were imposed during the optimization process. Vibrational frequencies were used to characterize stationary points as minima and to evaluate zero-point energies (ZPEs). Final energies for all structures **11A-13A** and **11B-13B** were calculated at the B3LYP/6-311+G(d,p) level of theory. Optimized geometries of all 2-iminothiazolidin-4-ones **11A-13A** (form **A**) and the corresponding tautomeric forms 2-aminothiazolidin-4-ones **11B-13B** (form **B**) are shown in Fig. 3.

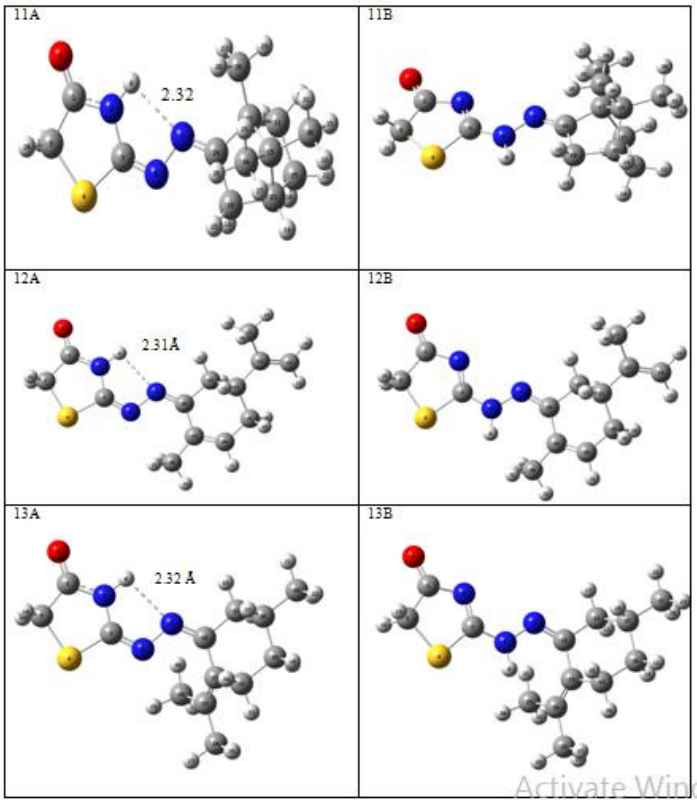


Figure 3 optimized geometries of two tautomeric forms: 2-iminothiazolidin-4-ones (**11A-13A**) and 2-aminothiazolidin-4-ones (**11B-13B**)

In table 1 we report the DFT calculated total energies and relative energies for **11A-13A** and their corresponding tautomeric forms **11B-13B**.

Table 1: Calculated total energies (au), total energies + zero-point energies ZPE (au), relative total energies (kcal/mol) with ZPE and without ZPE (kcal/mol) between the two tautomeric forms **A** and **B**

compound	energy	energy + ZPE	ΔE_{AB} [a]	$\Delta(E+ZPE)_{AB}$ [b]
11A	-1144.7103188	-1144.401400	8.989	8.778
11B	-1144.6959938	-1144.387410		
12A	-1143.476449	-1143.192128	10.247	10.018
12B	-1143.4601195	-1143.176163		
13A	-1144.7079507	-1144.400853	5.863	5.888
13B	-1144.6986061	-1144.391469		

[a] $\Delta E_{AB} = [E_{tot}(B)-E_{tot}(A)]$

[b] $\Delta(E+ZPE)_{AB} = [(E_{tot}+ZPE)(B)-(E_{tot}+ZPE)(A)]$

The DFT calculation at the B3LYP/6-311+G(d,p) level shows that the **11A-13A** compounds are more stable than their tautomeric forms **11B-13B** respectively by 8.989, 10.247 and 5.863 kcal/mol and the inclusion of ZPE correction in the relative energies does not change their stabilities (Table 1). We can state then that the tautomer **A** is thermodynamically more stable than tautomer **B**. Moreover, we can add that the **11A-13A** compounds are stabilized by intramolecular $N_{(2)}H_{(8)}\ldots N_{(7)}$ hydrogen bonds (Fig.3).

2.3 Biological evaluation.

The antimicrobial properties of the new sulfur and nitrogen containing heterocycles with monoterpenic skeleton and their corresponding monoterpenic thiosemicarbazones compounds **5**–**6, 8a-b, 11-12 and 14a-b** were tested. Antibacterial properties were evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) MB5393 [20], *A. baumanii*, *P.aureginosa PAO-1*, *K. pneumonia and E. coli*, (all clinical isolates from MEDINA's Culture Collection when no especified; Vicente et al. 2013), whereas antifungal properties against *Candida albicans* MY1055 [20] and *Aspergillus fumigatus* ATCC 46645 [21]. Briefly, the microorganisms were incubated with the extracts for 18–30 h at 37°C. Sample 1:10 or 2:25 dilutions were used depending on the target strain. The activities were measured by monitoring the absorbance differences at 600 nm between the final and the initial incubation times, except for *A. fumigatus* where the activity was scored by using resazurin, an oxidation–reduction indicator of the cell viability [21].

Regarding the antifungal activities (Table2), compounds **6**, **12**, and **8a** showed strong inhibition of *A. fumigatus_ATCC46645* growth with MIC value ranges of 20.6, 4.3 and 15.2 μg/mL respectively, while **5**, **11** and **3** showed weaker activities within an MIC range of 27–64 μg/mL. **6** was the most active compound against *C. albicans* with MIC values of 27.3 μg/mL.

Concerning antibacterial activity none of the compounds were actives when tested at concentrations up to 64 μg/mL.

Table 2 Antimicrobial and antifungal activities of thiosemicarbazones **5-7**, indazoles **8a-b** and thiazolidinones **11-14**.

	A. fumigatus _ATCC46645		*C. albicans MY1055*		*B.baumanii MB5973*	*P.aureginosa MB5919*	*E. coli MB2884*	*MRSA MB5393*	*K. pneumoniae ATCC700603*
Compound	MIC_{90} μg/mL	MIC_{50} μg/mL	MIC_{90} μg/mL	MIC_{50} μg/mL	MIC_{90} μg/mL	MIC_{90} μg/mL	MIC_{90} μg/mL	MIC_{90} μg/mL	MIC_{90} μg/mL
5	>64	**31,5**	>64	>64	>64	>64	>64	>64	>64
11	>64	**39,5**	>64	>64	>64	>64	>64	>64	>64
6	**64,0**	**20,6**	**64**	**27,3**	>64	>64	>64	>64	>64
12	>64	**4,3**	>64	>64	>64	>64	>64	>64	>64
8a	**42,7**	**15,2**	>64	**50,9**	>64	>64	>64	>64	>64
8b	>64	**27,8**	>64	>64	>64	>64	>64	>64	>64
14a	>64	>64	>64	>64	>64	>64	>64	>64	>64
14b	>64	>64	>64	>64	>64	>64	>64	>64	>64

Additionally, the cytotoxicity of the different compounds against the HepG2 cell line (hepatocellular carcinoma, ATCC HB 8065) was evaluated by a classical 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide reduction colorimetric assay, with the same incubation times and assay concentrations as used for the antibiotic evaluation [22].

III. Experimental

3.1 General.

All reagents and solvents were purchased from commercial sources (Aldrich, 112 Acros) and used as received. Melting points (mp) were determined using a capillary apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed on plates precoated with E. Merck silica gel 60 F254 0.25mm thick. HRMS were obtained on a Q-TOF micromass spectrometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ with a BrukerAC 500 instrument. Chemical shifts (δ) are expressed in parts per million (ppm) with TMS as the internal standard. The elemental analyses were carried out on a CHN2400 Perkin-Elmer analyser. The monoterpenic thiosemicarbazones (**5-7**) were prepared according to the reported method [11-12-13]. Calculations were performed using the Gaussian 09W program [18]. The geometries for all Structures presented here were optimized at the density functional theory (DFT) level by using the DFT (rb3lyp/6-311+G (d,p)).

The two diastereoisomers **8a** (3aR,6R) and **8b** (3aS,6R) were analyzed on a Trace GC Thermo Finnigan chromatograph equipped with FID, using capillary columns BP (25 m 0.25 mm, SGE). The column temperature was programmed from 170 to 230°C at a linear flow rate of 2°C/min. The initial and final holds up time were 2 and 0 min, respectively, while the injector and detector were set at 250°C and 250°C, respectively. A sample volume of 5.0 μL was injected onto the column in split mode (splitless ratio -1,0).

3.2 General synthetic procedure

3.2.1 Method 1

A mixture of thiosemicarbazone (1.5 mmol), ethyl 2-bromoacetate (0.24 mL, 1.5 mmol) and anhydrous sodium acetate (0.37 g, 4.5 mmol) in absolute ethanol (30 mL) was stirred until reflux; the mixture was stirred under the same conditions till the completion of the reaction (1–3 h), the progress of the reaction was monitored

by TLC. The reaction mixture was left to cool, poured into cold water, extracted with ethyl acetate and purified by chromatography (SiO_2, hexane/ethyl acetate gradient).

3.2.2 Method 2

A mixture of thiosemicarbazone (1 mmol) and chloroacetic acid (~0.1 g, 1 mmol) in 20 mL absolute ethanol was refluxed for about 4 h. After cooling, the formed thiazolidin-4-one was filtered off, dried and crystallized in the appropriate solvent.

3.3 Spectral data

3.3.1 (3aR,6R)-3,3,6-trimethyl-3,3a,4,5,6,7-hexahydro-2H-indazole-2-carbothioamide 8a

Yield: 64%; m.p:156 °C (Ethanol).

^{1}H NMR : 6.943 and 5.762 ppm (2H,s,=N**H** and –S**H**) ; 2.65 to 2.9 ppm (1H,m,-C_{3a}**H**-jonction) ; 2.31 to 2.43 ppm (3H,m,-C_7**H**$_2$-) ; 1.81and 1.639 to 1.671 ppm (6H,s ,gem dimethyl –C_3(C**H**$_3$)$_2$; 1.62 to 1.70 ppm and1,63 to 1,67 ppm (4H,m,--C_4**H**$_2$-C_5**H**$_2$-) ; 0,906 ppm (3H,d[7.25 MHz],C_8**H**$_3$).

^{13}C NMR: 176.24 ppm (-**C**=NH) ; 159.84 ppm (-**C**$_{7a}$=N) ; 67.67ppm (-**C**$_3$(CH$_3$)$_2$) ; 59.12 ppm (-C_{3a}H-jonction) ; 33.95 ppm (-**C**$_7$H$_2$-) ; 29.86 and 21.36 ppm (-**C**$_4$H$_2$-**C**$_5$H$_2$-) ; 28.63 ppm (-**C**$_6$H(CH$_3$)) ; 21.00 and 28.36 ppm (gem dimethyl –(**C**$_9$H$_3$ and –**C**$_{10}$H$_3$); 18.01 ppm (C$_8$H$_3$).

HRMS (TOF-MS ES+) (m/z) : 226.1371 $[M+H]^+$ and Calcd. mass 226.1378 $[M+H]^+$.

Anal. Calcd. for $C_{11}H_{19}N_3S$: C 58.63, H 8.50, N 18.65. Found: C 58.37, H 8.46, N 18.57.

3.3.2 (3aS,6R)-3,3,6-trimethyl-3,3a,4,5,6,7-hexahydro-2H-indazole-2-carbothioamide 8b

Yield: 20%; m.p:202 °C (Ethanol).

^{1}H NMR : 6.93 and 5.78 ppm (2H,s,=N**H** and –S**H**) ; 2.62 to 2.70 ppm (1H,m,-C_{3a}**H**-jonction) ; 2.32 to 2.34 ppm (3H,m,-C_7**H**$_2$-) ; 1.80 and 1.64 to 1.67 ppm (6H,s ,gem dimethyl –C_3(C**H**$_3$)$_2$; 1.62 to 1.70 ppm and1,64 to 1,67 ppm (4H,m,--C_4**H**$_2$-C_5**H**$_2$-) ; 0,89 ppm (3H,d[7.25 MHz],C_8**H**$_3$).

^{13}C NMR: 176.18 ppm (-**C**=NH) ; 159.81 ppm (-**C**$_{7a}$=N) ; 67.62 ppm (-**C**$_3$(CH$_3$)$_2$) ; 59.07 ppm (-C_{3a}H-jonction) ; 33.90 ppm (-**C**$_7$H$_2$-) ; 29.81 and 21.31 ppm (-**C**$_4$H$_2$-**C**$_5$H$_2$-) ; 28.59 ppm (-**C**$_6$H(CH$_3$)) ; 20.97 and 28.39 ppm (gem dimethyl –(**C**$_9$H$_3$ and –**C**$_{10}$H$_3$); 17.97 ppm (C$_8$H$_3$).

HRMS (TOF-MS ES+) (m/z) : 226.1371 $[M+H]^+$ and Calcd. mass 226.1378 $[M+H]^+$.

Anal. Calcd. for $C_{11}H_{19}N_3S$: C 58.63, H 8.50, N 18.65. Found: C 58.38, H 8.47, N 18.56.

3.3.3 (1R,4R)-2-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)hydrazonothiazolidin-4-one 11.

Yield: 82%; m.p:187°C (Ethanol).

^{1}H NMR : 10.71 ppm (1H,s,N**H**) ; 3.70 ppm (2H,s,-C**H**$_2$-S); 2.64 ppm (1H ,dd[J=17.7MHz, J=3.85 MHz],CH_2-C=N) and 2.15 ppm (1H,d[J=17.05 MHz], CH_2-C=N); 1.91ppm (1H,t[J=4.35MHz],-CH-) ; 0.94 and 1.05 ppm (6H,s,gem CH_3) ; 0.82 ppm (3H,s,-CH_3) ; 1.23 to 1.88 (4H,m,2-CH_2- CH_2).

^{13}C NMR : 180.28 ppm (-C=O); 174.28 ppm (-C=N-) ; 163.06 ppm (-S-C=N) ; 53.28 ppm (-C-(CH$_3$)$_2$) ; 48.03 ppm (-CH-) ; 35.84 ppm (-CH_2-C=N-) ; 33.22 ppm (-CH_2-S) ; 32.87 and 27.20 ppm (-CH_2-CH_2-) ; 19.65 ppm (-CH_3) ; 18.85 and 11.19 ppm (gem –CH_3).

HRMS (TOF-MS ES+) (m/z) : 266.1326 $[M+H]^+$ and Calcd. mass 266.1327 $[M+H]^+$.

Anal. Calcd. for $C_{13}H_{19}N_3OS$: C 58.84, H 7.22, N 15.83. Found: C 58.66, H 7.20, N 15.79.

3.3.4 2-(((R)-2-methyl-5-(prop-1-en-2-yl)cyclohex-2-enylidene)hydrazono)thiazolidin-4-one 12.

Yield: 86%; m.p:149°C (Ethanol).

^{1}H NMR : 10.53 ppm (1H,s,NH) ;6.21 ppm (1H,m,-CH=C-) ; 4.73 ppm (2H,d,CH_2=C of prop-1-en-2-yl) ; 3.74 ppm (2H,s,CH_2-S); 2.39 ppm (1H,m,CH_2-CH-CH_2) ; from 2.10 to 2.32 ppm and 3.17 to 3.22 ppm(4H,m,two –CH_2- of cycle carvone).

^{13}C NMR : 174.00 ppm (-C=O) ; 163.98 ppm (--C=N-); 163.16 ppm (-S-C=N) ; 147.99 ppm (CH_2=C-(CH)(CH_3); 135.97 ppm (-CH=C(CH_3) ; 133.27 ppm (=C(CH_3)) ; 110.12 ppm (CH_2=C(CH_3) ; 41.36 ppm (CH_2-CH-CH_2) ; 33.15 ppm (-CH_2- S) ; 31.03 and 30.99 ppm (two –CH_2- of cycle carvone); 20.63 and 17.84 ppm (tow methyl of carvone).

HRMS (TOF-MS ES+) (m/z) : 264.1178 $[M+H]^+$ and Calcd. mass 264.1171 $[M+H]^+$.

Anal. Calcd. for $C_{13}H_{17}N_3OS$: C 59.29, H 6.51, N 15.96. Found: C 59.28, H 6.55, N 15.93.

3.3.5 2-(((R)-5-methyl-2-(propan-2-ylidene)cyclohexylidene)hydrazono)thiazolidin-4-one 13.

Yield: 9%; oil

^{1}H NMR: 8.69 ppm (1H, s, -NH-); 3.71ppm (2H, s, -CH_2-S); 2.68 ppm (1H, m) and 1.93 ppm (1H, m) (H_2-C_3-); 2.56 ppm (1H, m) and 1.89 ppm (1H, m) (H_2-C_6); 1.87 ppm (1H, m, H-C_5); 1.79 ppm (1H, m) and 1.12

ppm (1H, m) (H_2-C_4); 1.67 ppm (3H, s, CH3-C=); 1.45 ppm (3H, s, -CH_3-C=); 0.97 ppm (3H, d [J=6,0 Hz], -CH3-C_5).
^{13}C NMR: 178.45 ppm (-$C_{4'}$=O); 170.16 ppm (-S-$C_{2'}$=N) ; 166.71 ppm (-N =C_1); 130.34 and 128.79 ppm (C_2=C-); 45.21ppm (H_2-C_6); 35.78 ppm (H_2-C_4); 35.55 ppm (H-C_5); 34.86 ppm (-$H_2C_{5'}$- S-); 30.76 ppm (H_2-C_3); 23.30 ppm (-CH_3); 22.18 ppm (-CH_3); 19.44 ppm (-CH_3).
HRMS (TOF-MS ES+) (m/z) : 265.1271 $[M+H]^+$ and Calcd. mass 265.1278 $[M+H]^+$.
Anal. Calcd. for $C_{13}H_{19}N_3OS$: C 58.84, H 7.22, N 15.83. Found: C 58.81, H 7.25, N 15.82.

3.3.6 2-((3aR,6R)-3,3,6-trimethyl-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)thiazol-4(5H)-one 14a
Yield: 90% (from **8a**); m.p: 210°C (Ethanol).
^{1}H NMR: 3.77 ppm ($C_{5'}H_2$ thiazolidinone) ; 2.77 ppm (1H,m,-CH-jonction) ; 2.41 ppm (1H,m,C_6H)) ; 1.54 and 1.69 ppm (6H,s ,gem dimethyl –$C_3(CH_3)_2$; 1.08 ppm (3H,d[7.20 MHz],C_8H_3).
^{13}C NMR: 188.48 ppm(-$C_{4'}$=O); 177.202 ppm (-S-$C_{2'}$=N-);165.67 ppm (-C=N-) ; 66.56 ppm (-$C_3(CH_3)_2$; 58.88 ppm (-C_{3a}H-jonction) ; 38.09 ppm (-CH_2- S-); 33.12 ppm (-C_7H_2-) ; 29.60 and 21.31 ppm (-C_4H_2-C_5H_2-) ; 28.50 ppm (-$C_6H(CH_3)$) ; 28.50 and 20.33 ppm (gem dimethyl –$C(C_9H_3)$ and –$C(C_{10}H_3)$.
HRMS (TOF-MS ES+) (m/z): 266.1320 $[M+H]^+$ and Calcd. mass 266.1327 $[M+H]^+$.
Anal. Calcd. for $C_{13}H_{19}N_3OS$: C 58.84, H 7.22, N 15.83. Found: C 58.80, H 7.26, N 15.80.

3.3.7 2-((3aS,6R)-3,3,6-trimethyl-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)thiazol-4(5H)-one 14b
Yield: 90% (from **8b**); m.p:210°C (Ethanol).
^{1}H NMR; 3.77 ppm ($C_{5'}H_2$ thiazolidinone) ; 2.77 ppm (1H,m,-CH-jonction) ; 1.95 ppm (3H,m,C_6H)) ; 1.53 and 1.67 ppm (6H,s ,gem dimethyl –$C_3(CH_3)_2$; 0.95 ppm (3H,d[7.20 MHz],C_8H_3).
^{13}C NMR: 188.48 ppm (-C=O) ;177.202 ppm (-S-$C_{2'}$=N-);166.86 ppm (-C=N-) ; 67.62 ppm (-$C_3(CH_3)_2$; 59.08 ppm (-C_{3a}H-jonction) ; 38.09 ppm (-CH_2- S-) ;33.12 ppm (-C_7H_2-); 29.60 and 22.05 ppm (-C_4H_2-C_5H_2-); 28.50 ppm (-$C_6H(CH_3)$) ; 29.60 and 20.34 ppm (gem dimethyl –$C(C_9H_3)$ and –$C(C_{10}H_3)$.
HRMS (TOF-MS ES+) (m/z) : 266.1320 $[M+H]^+$ and Calcd. mass 266.1327 $[M+H]^+$.
Anal. Calcd. for $C_{13}H_{19}N_3OS$: C 58.84, H 7.22, N 15.83. Found: C 58.80, H 7.24, N 15.81.

IV. Conclusion

In summary, we have described the synthesis of five new sulfur and nitrogen containing heterocyclic compounds having monoterpenic skeleton. Four of the resulting thiazolidin-4-ones were obtained via heterocyclisation reactions using chloracetic acid or ethyl bromoacetate. An indazole derivative was obtained from acid or base catalyzed rearrangement of thiosemicarbazone pulegone. All these newly prepared heterocyclic compounds were fully characterized using spectroscopic data and their stabilities were studied using DFT calculations. They showed that the tautomeric form **A** is thermodynamically favorably than this corresponding to the tautomeric form **B**. Furthermore, their antibacterial and antifungal activities were determined resulting some of them were active at against two fungal strains.

References

[1]. Archana , Srivastava, V.K.; Kumar, A, Synthesis of newer thiadiazolyl and thiazolidinonyl quinazolin-4(3H)- ones as potential anticonvulsant agents, *European Journal of Medicinal Chemistry*, *37*, 2002, , 873-882.
[2]. Barreca, M.L.; Chimirri, A.; De Luca, L.; Monforte, A.M.; Monforte, P.; Rao, A.; Zappala, M.; Balzarini, J.; De Clercq, E.; Pannecouque, C.; Witvrouw, M, Discovery of 2,3-Diaryl-1,3-thiazolidin-4-ones as Potent Anti-HIV-1 Agents, *Bioorganic & Medicinal Chemistry Letters. 11*, 2001, 1793-1796.
[3]. Rao, A.; Balzarini, J.; Carbone, A.; Chimirri, A.; De Clercq, E.; Monforte, A.M.; Monforte, P.; Pannecouque, C.; Zappalà, M, Synthesis of new 2,3-diaryl-1,3-thiazolidin-4-ones as anti-HIV agents, *IL. Farmaco. 59*, 2004, 33-39.
[4]. Rao, A.; Chimirri, A.; Ferro, S.; Monforte, A.M.; Monforte, P.; Zappalà, M, Microwave-assisted synthesis of benzimidazole and thiazolidinone derivatives as HIV-1 RT inhibitors, *Arkivoc. V*, 2004, 147-155.
[5]. Bhoot, D.P.; Khunt, R.C.; Shankhavara, V.K.; Parekh, H.H, Synthesis of Some New Heterocyclic Compounds with Potential Biological Activity, *Journal of Sciences, Islamic Republic of Iran, 17(4*), 2006, 323-325.
[6]. Altintaş, H.; Ateş, Ö.; Birteksöz, S.; Ötük, G.; Uzun, M.; Şanata, D., Synthesis of Mannich Bases of Some 2,5-Disubstituted 4-Thiazolidinones and Evaluation of Their Antimicrobial Activities, *Turkish Journal of Chemistry, 29*, 2005, 425-435.
[7]. Desai, K.G.; Desai, K.R., A facile microwave enhanced synthesis of sulfur-containing 5-membered heterocycles derived from 2-mercaptobenzothiazole over ZnCl 2/DMF and antimicrobial activity evaluation, *Journal of Sulfur Chemistry, 27(4)*, 2006, 315-328.
[8]. Vazzana, I.; Terranova, E.; Mattioli, F, Aromatic Schiff bases and 2,3-disubstituted-1,3-thiazolidin-4-one derivatives as antiinflammatory agents, *Arkivoc. V*, 2004, 364-374.
[9]. Kumar, A.; Rajput, C.S.; Bhati, S.K, Synthesis of 3-[40-(p-chlorophenyl)-thiazol-20-yl]-2-[(substituted azetidinone/thiazolidinone)-aminomethyl]-6-bromoquinazolin-4- ones as anti-inflammatory agent, *Bioorganic & Medicinal Chemistry, 15*, 2007, 3089-3096.
[10]. Feddouli, A.; Ait Itto, My. Y.; Hasnaoui, A.; Villemin, D.; Jaffrès, P.A.; Santos, J. S. O.; Riahi, A.; Huet, F.; Daran, J.C, One Pot Diastereoselective Synthesis of New Chiral Spiro- 1,3,4-thiadiazoles and 1,4,2-Oxathiazoles from (1R)-Thiocamphor , *Journal of Heterocyclic Chemistry, 41*, 2004, 731-735.
[11]. Brousse, B.N.; Moglioni, A.G.; Alho, M.M.; Larena, Á.Á.; Moltrasio, G.Y.; D´Accorsob, N.B, Behavior of thiosemicarbazones derived from some terpenones under acetylating conditions, *Arkivoc. X*, 2002, 14-23.

[12]. García, C.C.; Brousse, B.N.; Carlucci, M.J.; Moglioni, A.G.; Alho, M.M.; Moltrasio, G.Y.; D´Accorso, N.B.; Damonte, E.B, Inhibitory effect of thiosemicarbazone derivatives on Junin virus replication in vitro, *Antiviral Chemistry & Chemotherapy, 14*, 2003, 99-105.
[13]. Choudhary, A.; Sharma, R.; Nagara, M, Synthesis, characterization and antimicrobial activity of mixed ligand complexes of Co (II) and Cu (II) with N, O/S donor ligands and amino acids, *Iternational Research Journal of Pharmacy and Pharmacology, 1(6)*, 2001, 172-187.
[14]. Mohamed, S.F.; Youssef, M.M.; Amr, A.E.; Kotb, E.R, Antimicrobial Activities of some Synthesized Pyridines, Oxazines and Thiazoles from 3-Aryl-1-(2-naphthyl)prop-2-en-1-ones, *Scientia Pharmaceutica, 76*, 2008, 279-303.
[15]. Abdul Nasser, A.J.; Idhayadhulla, A.; Kumar, R.S.; Selvin, J, Synthesis of Some 2-Thioxo-imidazolidin-4-one Derivatives and its Antimicrobial Activity, *E-Journal of Chemistry, 7(4)*, 2010, 1320-1325.
[16]. N'ait Ousidi, A.; Ait Itto, My. Y.; Auhmani, A.; Riahi, A.; Daran, J.C.; Auhmani, A, Crystal structure of 2-[(3aS,6R)-3,3,6-trimethyl- 3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl]thiazol- 4(5H)-one, *Acta Crystallographica, E72*, 2016, 334-336.
[17]. Tripathi, A.C.; Gupta, S.J.; Fatima, G.N.; Sonar, P.K.; Verma, A.; Saraf, S.K, 4-Thiazolidinones: The advances continue, *European Journal of Medicinal Chemistry, 72*, 2014, 52-77.
[18]. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G.A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H.P.; Izmaylov, A.F.; Bloino, J.; Zheng, G.; Sonnenberg, J.L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. A.; Peralta, J.E.; Ogliaro, F.; Bearpark, M.; Heyd, J.J.; Brothers, E.; Kudin, K.N.; Staroverov, V.N.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, J.C.; Iyengar, S.S.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, J.M.; Klene, M.; Knox, J.E.; Cross, J.B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R.E.; Yazyev, O.; Austin, A.J.; Cammi, R.; Pomelli, C.; Ochterski, J.W.; Martin, R.L.; Morokuma, K.; Zakrzewski, V.G.; Voth, G.A.; Salvador, P.; Dannenberg, J.J.; Dapprich, S.; Daniels, A.D.; Farkas, O.; Foresman, J.B.; Ortiz, J.V.; Cioslowski, J.; Fox.D.J.Gaussian 09, Revision A.02; Gaussian, Inc.: Wallingford, CT, 2009.
[19]. (a) Becke, A. D, Density-functional thermochemistry. III. The role of exact Exchange, *Journal. Chemical Physics, 98*,1993, 5648-5652. (b) Lee, C.; Yang, W.; Parr, R. G, Development of the Colic-Salvetti correlation-energy formula into a functional of the electron density, *Physical Review, B 37*, 1988, 785-789.
[20]. De la Cruz, M.; Martín, J .; González-Menéndez, V.; Pérez-Victoria, I.; Moreno, C.; Tormo, J.R.; El Aouad, N.; Guarro, J.; Vicente, F.; Reyes, F.; Bills, G.F, Chemical and Physical Modulation of Antibiotic Activity in Emericella Species, *Chemistry & Biodiversity, 9*, 2012, 1095-1113.
[21]. Monteiro, M.C.; De la Cruz, M.; Cantizani, J.; Moreno, C.; Tormo, J.R.; Mellado, E.; De Lucas, J.R.; Asensio, F.; Valiante, V.; Brakhage, A.A.; Latgé, J.P.; Genilloud, O.; Vicente, F, A New Approach to Drug Discovery: High-Throughput Screening of Microbial Natural Extracts against Aspergillus fumigatus Using Resazurin, *Journal of Biomolecular Screening, 17*, 2012, 542-549.
[22]. Zhang, L.; Ravipati, A. S.; Koyyalamudi, S.R.; Jeong, S. C.; Reddy, N.; Bartlett, J.; Smith, P. T.; De la Cruz, M.; Monteiro, M. C.; Melguizo, A.; Jiménez, E.; Vicente, F, Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs, *Asian Pacific Journal of Tropical Medicine, 6(9)*, 2013, 673-681.